EP 04/11567

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The Patent Office

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
South Wales NP10 8QQ

1.	Your reference	4-33395P1
2.	Patent application number *(The Patent Office will fill in this part)*	0324210.4
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND
	Patent ADP number *(if you know it)*	07125487005
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND
4.	Title of invention	Organic Compounds
5.	Name of your agent *(If you have one)*	Craig McLean
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Novartis Pharmaceuticals UK Limited Patents and Trademarks Wimblehurst Road Horsham, West Sussex RH12 5AB
	Patents ADP number *(if you know it)*	07181522002 ✓

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing (day/month/year)

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day/month/year)

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *(see note (d))*	Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	8
Claim(s)	3
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature — Date

Craig McLean — 15th October 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr. Trevor Drew — 01403 323069

Warning

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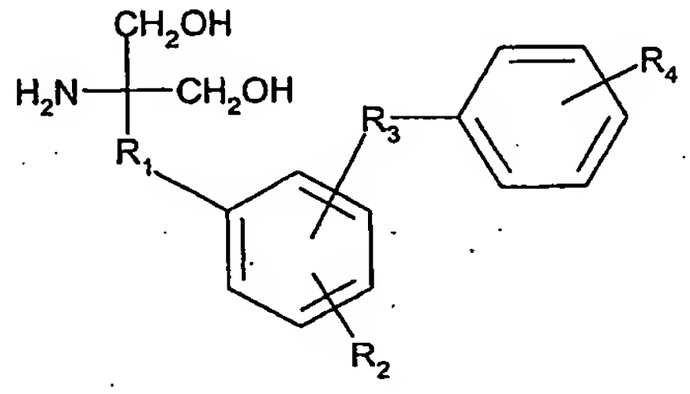
Notes

Organic Compounds

The present invention relates to organic compounds and their use as pharmaceuticals, a process for preparing such compounds and to intermediate compounds useful in such a process.

More particularly, the invention relates to a compound of formula I:



I

wherein:

R_1 is C_{2-8}-alkylene;

R_2 is C_{1-20}-alkyl, optionally substituted by halogen;

R_3 is C_{2-8}-alkylene; and

R_4 is H or C_{1-20}alkyl, optionally substituted by halogen;

in free or salt form.

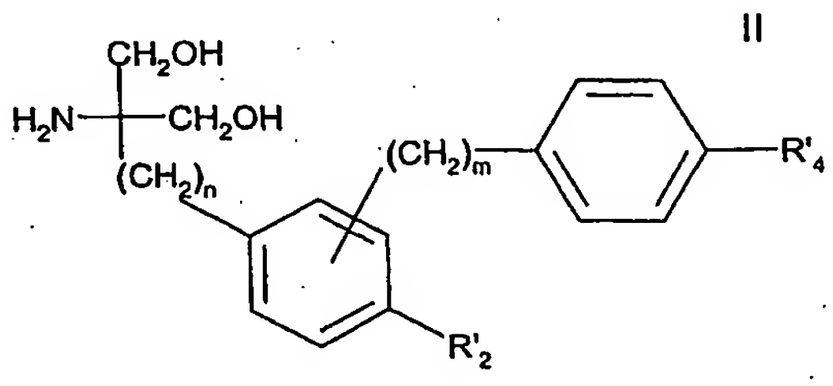
Alkyl means straight or branched alkyl.

Preferred compounds of formula I are those wherein R_1 and/or R_3 is C_{1-4}alkylene, e.g. ethylene. R_3 may be in ortho, meta or para, preferably in meta or ortho.

R_2 is preferably C_{6-14}-alkyl, e.g. octyl, optionally substituted by halogen. R_2 may be in ortho, meta or para, preferably in para.

R_4 is preferably H or C_{6-14}-alkyl, e.g. octyl, optionally substituted by halogen. R_4 may be in ortho, meta or para, preferably in para.

Particularly preferred compounds are those of formula II:



II

wherein

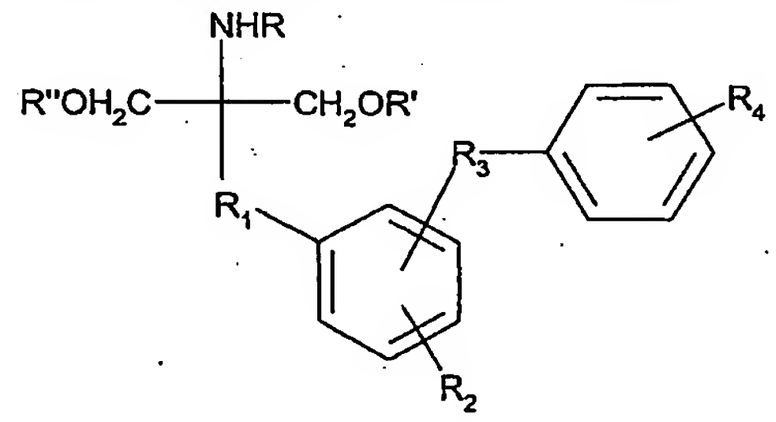
n is an integer from 1 to 4, e.g. 2;

m is an integer from 2 to 4, e.g. 2;
R'_2 is C_{6-14}-alkyl, e.g. octyl; and
R'_4 is H or C_{6-14}-alkyl, e.g. H or octyl;
in free or salt form.

The compounds of the invention may exist in free form or in salt form, e.g. addition salts with e.g. organic or inorganic acids, for example trifluoroacetic acid or hydrochloride acid.

The present invention is to be understood as embracing the optical isomers as well as racemates and mixtures of the compounds of formula I. Compounds of formula I and salts of the present invention encompass hydrate and solvate forms.

A compound of formula I may be prepared by deprotecting a compound of formula III:



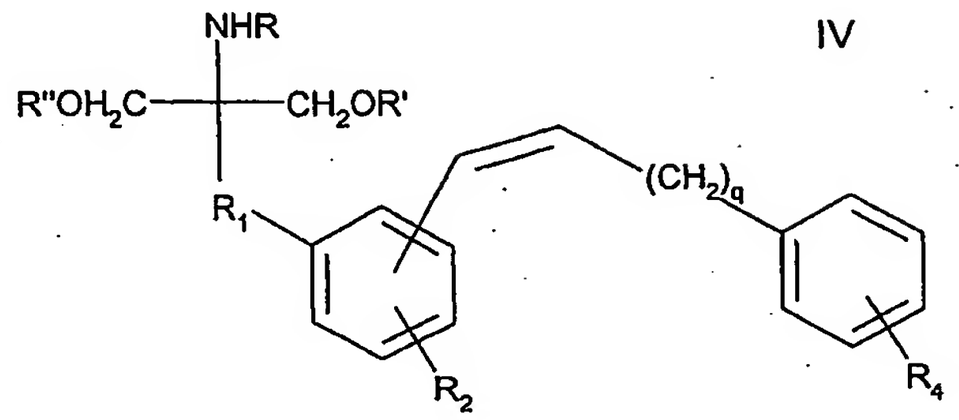
III

wherein R_1, R_2, R_3 and R_4 are as defined in formula I, and each of R, R' and R" is a protecting group; and recovering the resulting compound of formula I in free or salt form.

The process may be performed according to methods known in the art, for example by base- or acid-catalysed hydrolysis, e.g. as described in the examples.

Protecting groups, their introduction and removal are described, for example, in "Protective Groups in Organic Synthesis", T. W. Greene et al., John Wiley & Sons Inc., Second Edition 1991. Preferably each protecting group, e.g. the amino protecting group R and/or one or both of the hydroxy protecting groups R' and R", is acyl, e.g. a residue R_y-CO- wherein R_y is C_{1-6}alkyl, C_{3-6}cycloalkyl, phenyl or phenyl-C_{1-4}alkyl, e.g. acetyl.

Where required, the compound of formula I obtained in free form may be converted into the



IV

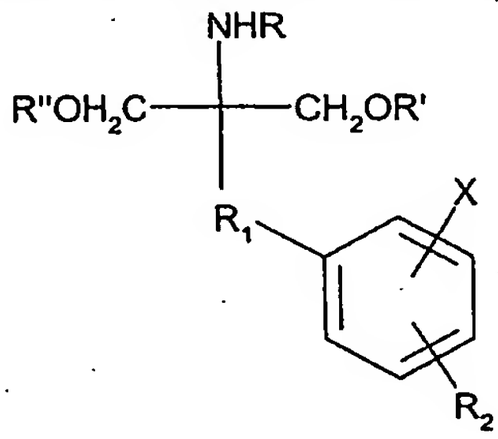
wherein

R, R', R'', R_1, R_2 and R_4 are as defined above; and

q is an integer from 0 to 6;

using known methods, e.g. reduction with hydrogen and a palladium catalyst. The compound of formula IV may be in the cis or trans form.

A compound of formula IV may be prepared by reacting a compound of formula V:

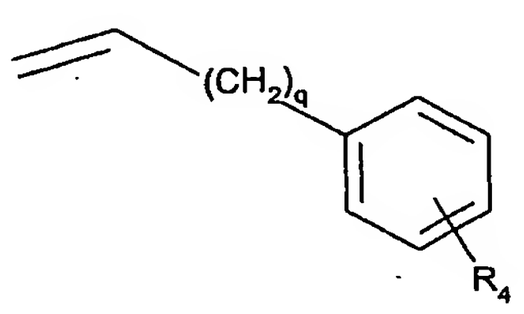


V

wherein R, R', R'', R_1 and R_2 are as defined above; and

X is halogeno, e.g. bromo;

with a compound of formula VI:



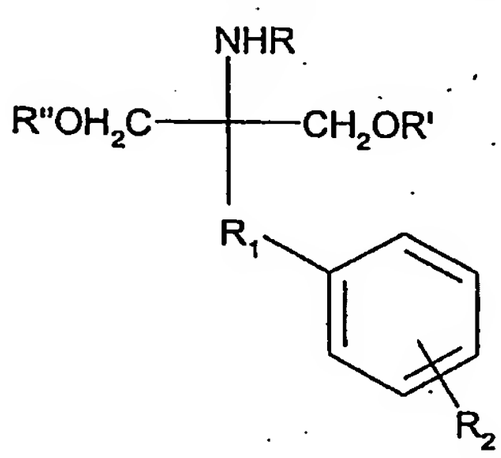
VI

wherein q and R_4 are as defined above;

e.g. using known methods, for example by Heck coupling.

Preferred compounds of formulae V and VI are those which may be used to produce a compound of formula II, i.e. compounds of formula V wherein R_1 is C_{1-4}alkylene and R_2 is in the position para and is equal to R'_2 as defined in formula II, and compounds of formula VI wherein R_4 is in the position para and is equal to R'_4 as defined in formula II.

A compound of formula V may be prepared by halogenating, e.g. brominating with a brominating agent, e.g. HBr or Br_2, a compound of formula VII:



VII

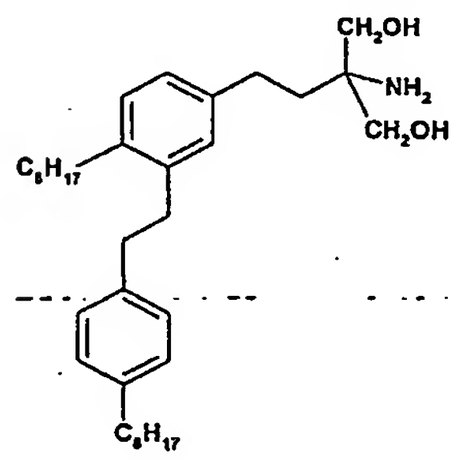
wherein R, R', R'', R_1 and R_2 are as defined above. Isomers of compounds of formula V formed by this process, in which the halogen atom is attached to the benzene ring at alternative positions, may be separated by standard procedures, e.g. column chromatography.

The compounds of formulae III to V are intermediates useful in the production of the compounds of formulae I and II and also form part of the present invention. The compounds formulae VI and VII are known or may be produced in accordance with known procedures.

The following non-limiting examples illustrate the invention.

Example 1

2-Amino-2-(2-{4-octyl-3-[2-(4-octyl-phenyl)-ethyl]-phenyl}-ethyl)-propane-1,3-diol



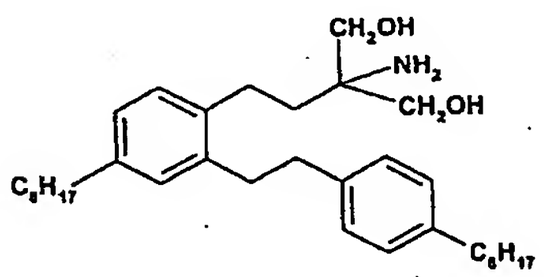
a) 10mmol of acetic acid 2-acetoxymethyl-2-acetylamino-4-(4-octyl-phenyl)-butyl ester is dissolved in acetonitrile at room temperature in a three-necked flask. 1 mg of iodine is

b) 10mmol of acetic acid 2-acetoxymethyl-2-acetylamino-4-(3-bromo-4-octyl-phenyl)-butyl ester is dissolved in dimethylformamide, 1 equivalent of 1-octyl-4-vinyl-benzene is added and the reaction vessel is purged with Ar. Then 5 mol% tetrakistriphenylphosphin-palladium is added and the temperature is raised to 80 -120 °C. When the reaction is completed, the product is precipitated by the addition of water. Acetic acid 2-acetoxymethyl-2-acetylamino-4-{4-octyl-3-[2-(4-octyl-phenyl)-vinyl]-phenyl}-butyl ester is purified by column chromatography.

c) 10mmol of the purified compound are dissolved in ethanol, Raney nickel is added and hydrogenated with H_2 until one equivalent is taken up. Then water and sodium hydroxide solution are added and the mixture is stirred until the hydrolysis of the acetyl groups is completed. If necessary the mixture is slightly heated. 2-Amino-2-(2-{4-octyl-3-[2-(4-octyl-phenyl)-ethyl]-phenyl}-ethyl)-propane-1,3-diol is isolated by neutralization of the reaction mixture and dilution with water and purified by column chromatography.

Example 2

2-Amino-2-(2-{4-octyl-2-[2-(4-octyl-phenyl)-ethyl]-phenyl}-ethyl)-propane-1,3-diol



The compound above is prepared by a process analogous to that described in example 1, but wherein 2-acetoxymethyl-2-acetylamino-4-(2-bromo-4-octyl-phenyl)-butyl ester is used in step b) instead of the corresponding 3-bromo-substituted compound.

The compounds of formula I, in free form or in pharmaceutically acceptable salt form, exhibit valuable pharmacological properties, e.g. agonism of S1P receptors, e.g. as indicated in in vitro and in vivo tests and are therefore indicated for therapy.

A. Binding affinity of S1P receptor agonists to individual human S1P receptors

Transient transfection of human S1P receptors into HEK293 cells

S1P receptors and G_i proteins are cloned, and equal amounts of 4 cDNAs for the EDG receptor, G_i-α, G_i-β and G_i-γ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977;11;223 and DS. Im et al., Mol. Pharmacol. 2000;57;753). Briefly, a DNA mixture containing 25 µg of DNA and

0.25 M CaCl is added to HEPES-buffered 2 mM Na_2HPO_4. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 h, the monolayers are washed with phosphate-buffered saline and refed media (90% 1:1 Dulbecco's modified essential media (DMEM):F-12 + 10% fetal bovine serum). The cells are harvested 48-72 h after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 $MgCl_2$, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800×g, the supernatant is diluted with HME without sucrose and centrifuged at 100,000×g for 1h. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000×g. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by immersion in liquid nitrogen. The membranes are stored at 70°C. Protein concentration is determined spectroscopically by Bradford protein assay.

<u>GTPγS binding assay using S1P receptor/HEK293 membrane preparations</u>

GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000; 57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 $MgCl_2$, pH 7.5) using 25 µg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 µM GDP and 0.1 nM [^{35}S]GTPγS (1200 Ci/mmol) and incubated at 30°C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

The compounds of formula I are therefore useful as sphingosine-1 phosphate (S1P) receptor agonists or antagonists for:

a) treatment and prevention of organ or tissue-transplant rejection, for example for the treatment of the recipients of heart, lung, combined heart-lung, liver, kidney, pancreatic, skin or corneal transplants, and the prevention of graft-versus-host disease, such as sometimes occurs following bone marrow transplantation; particularly in the treatment of acute or chronic allo- and xenograft rejection or in the transplantation of insulin producing cells, e.g.

rhinitis/conjunctivitis, allergic contact dermatitis, inflammatory diseases optionally with underlying aberrant reactions, e.g. inflammatory bowel disease, Crohn's disease or ulcerative colitis; intrinsic asthma, inflammatory lung injury, inflammatory liver injury, inflammatory glomerular injury, atherosclerosis, osteoarthritis, irritant contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, cutaneous manifestations of immunologically-mediated disorders, inflammatory eye disease, keratoconjunctivitis, myocarditis or hepatitis.

For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained at daily dosages of from about 0.1 to about 100 mg/kg body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range of from about 0.5 mg to 2000 mg, conveniently administered, for example, in divided doses up to four times a day or in retard form.

The compounds of formula I may be administered by any appropriate route, e.g. orally, for example in the form of a tablet or capsule, topically or parenterally, for example intravenously. Pharmaceutical compositions comprising a compound of formula I in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutically acceptable carrier or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier or diluent. Unit dosage forms for oral administration contain, for example, from about 0.1 mg to about 500 mg of active substance.

Compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form, e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

The compounds of formula I may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents e.g. for the treatment or prevention of allograft acute or chronic rejection or inflammatory or autoimmune disorders. For example, they may be used in combination with calcineurin inhibitors, e.g. cyclosporin A, cyclosporin G, FK-506, ABT-281, ASM 981; an mTOR inhibitor, e.g. rapamycin, 40-O-(2-hydroxy)ethyl-rapamycin, CCI779, ABT578 or AP23573 etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; another S1P receptor agonist, e.g. FTY 720 or an analogue thereof; leflunomide or analogs thereof; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or analogs thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD 11a/CD18, CD7, CD25, CD 27, B7, CD40, CD45,

CD58, CD 137, ICOS, CD150 (SLAM), OX40, 4-1BB or their ligands, e.g. CD154; or other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y, or other adhesion molecule inhibitors, e.g. mAbs or low molecular weight inhibitors including LFA-1 antagonists, Selectin antagonists and VLA-4 antagonists.

Where a compound of formula I is administered in conjunction with another immunomodulating or anti-inflammatory agent, dosages of the co-administered immunomodulating or anti-inflammatory agent will of course vary depending on the type of co-drug employed, on the condition to be treated and so forth.

The present invention thus provides:

1. A method of treating or preventing organ or tissue transplant rejection, comprising administering to a subject a therapeutically effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof.

2. A method of treating or preventing an autoimmune disease or inflammatory condition, comprising administering to a subject a therapeutically effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof.

3. A compound of formula I, or a pharmaceutically acceptable salt thereof, for use as a pharmaceutical.

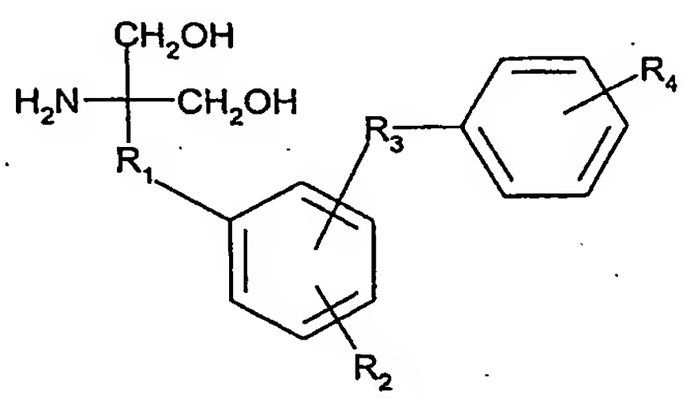
4. A pharmaceutical composition comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent or carrier.

5. Use of a compound of formula I, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament, e.g. in a method as disclosed above.

6. A pharmaceutical combination comprising (a) a compound of formula I and (b) a second drug substance, said second drug substance being suitable for the prevention or treatment

Claims

1. A compound of formula I:



I

wherein:

R_1 is C_{2-8}-alkylene;

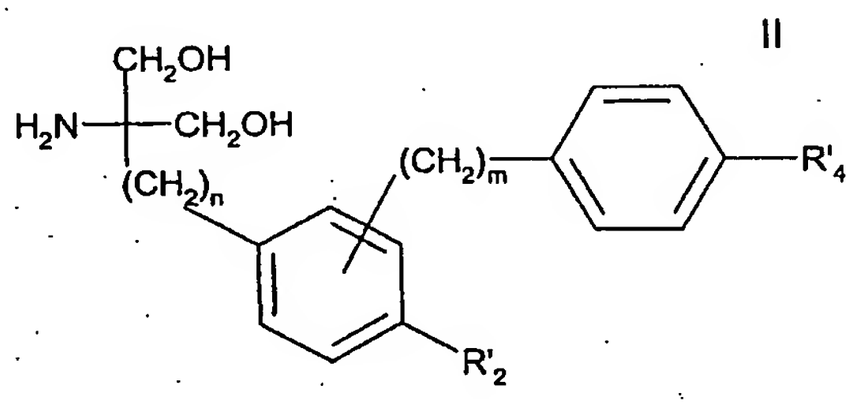
R_2 is C_{1-20}-alkyl, optionally substituted by halogen;

R_3 is C_{2-8}-alkylene; and

R_4 is H or C_{1-20}alkyl, optionally substituted by halogen;

in free or salt form.

2. A compound according to claim 1, wherein the compound is of formula II:



II

wherein

n is an integer from 1 to 4;

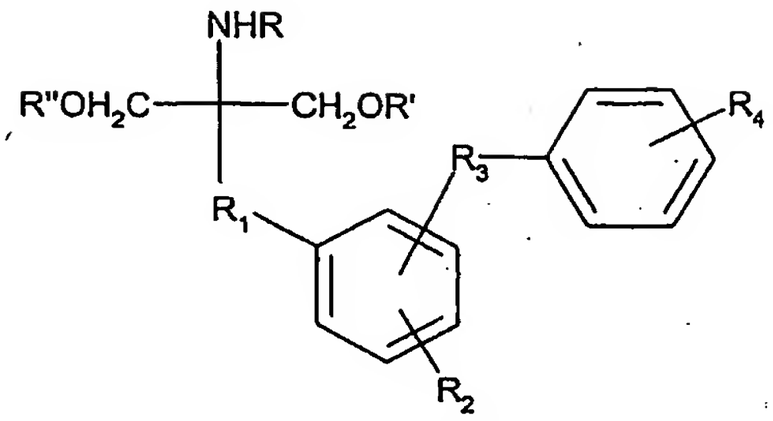
m is an integer from 2 to 4;

R'_2 is C_{6-14}-alkyl; and

R'_4 is H or C_{6-14}-alkyl;

in free or salt form.

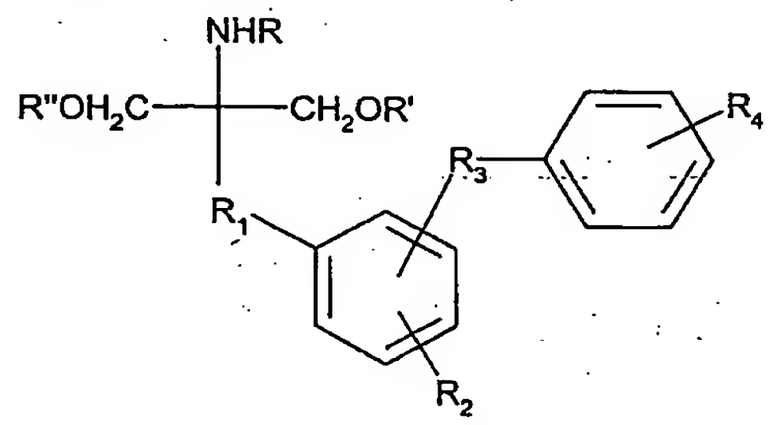
3. A process for producing a compound according to claim 1, comprising deprotecting a compound of formula III:



III

wherein R_1, R_2, R_3 and R_4 are as defined in claim 1, and each of R, R' and R" is a protecting group; and recovering the resulting compound of formula I in free or salt form.

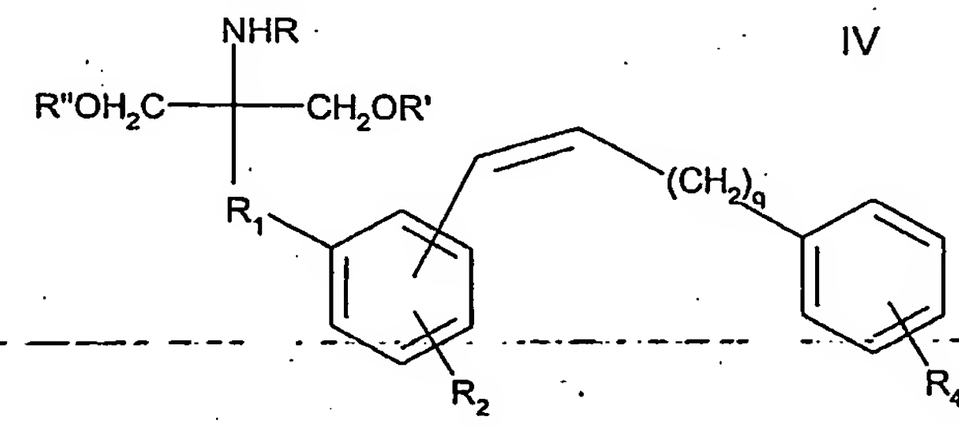
4. A compound of formula III:



III

wherein R_1, R_2, R_3 and R_4 are as defined in claim 1, and each of R, R' and R" is a protecting group, in free or salt form.

5. A compound of formula IV:



IV

wherein R, R', R", R_1, R_2 and R_4 are as defined in claims 1 and 4, and q is an integer from 0 to 6, in free or salt form.

6. A pharmaceutical composition comprising a compound according to claim 1, in free or

8. Use of a compound according to claim 1, in free or pharmaceutically acceptable salt form, as a pharmaceutical.

9. Use of a compound according to claim 1, in free or pharmaceutically acceptable-salt form, for the preparation of a medicament for preventing or treating organ or tissue transplant rejection, or an autoimmune disease or inflammatory condition.

PCT/EP2004/011567